

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A23B 4/20, A23L 3/3472, 3/3535</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/33391</b>
			<b>(43) International Publication Date:</b> 6 August 1998 (06.08.98)
<b>(21) International Application Number:</b> PCT/US98/01784		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
<b>(22) International Filing Date:</b> 30 January 1998 (30.01.98)		<b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(30) Priority Data:</b> 60/036,654                      30 January 1997 (30.01.97)                      US 09/015,388                      29 January 1998 (29.01.98)                      US			
<b>(71) Applicant:</b> KOCH ENTERPRISES, INC. [US/US]; 4111 East 37th Street North, Wichita, KS 67201 (US).			
<b>(72) Inventor:</b> WHITE, Richard, A.; 336 Green Valley Road, Andover, KS 64002 (US).			
<b>(74) Agents:</b> HERMAN, Joan, Optican et al.; Shook, Hardy & Bacon L.L.P., One Kansas City Place, 1200 Main Street, Kansas City, MO 64105-2118 (US).			
<b>(54) Title:</b> ANTIMICROBIAL COMPOSITION AND METHOD FOR FOOD PRODUCTS			
<b>(57) Abstract</b>  An antimicrobial composition and method for producing an antimicrobial effect on food products by inhibiting the growth of pathogenic bacteria and food spoilage bacteria. The composition has an active ingredient which is a mustard seed or its derivative, or the synthetic equivalent thereof. The composition is added directly to food products such raw or uncooked meats to achieve an antimicrobial effect by reducing and controlling bacteria growth, without adversely affecting the appearance, taste, or texture of the meat.			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

**ANTIMICROBIAL COMPOSITION AND METHOD FOR FOOD PRODUCTS**

This application claims the benefit of U.S. Provisional Application No. 60/036,654, filed January 30, 1997.

**Field of the Invention**

5           This invention relates in general to additives for meat, poultry and other food products, and, more particularly, to antimicrobial compositions and methods for adding antimicrobial compositions to meat and poultry products.

**Background of the Invention**

10           Bacterial contamination is an inherent consequence of producing and processing some food products, including among others, raw meat, poultry and fish products. Two categories of bacteria commonly encountered in food products are pathogens and spoilage flora. It is essential that harmful amounts and/or harmful species of bacteria not be present in food, often requiring some type of antimicrobial action to be taken in order to kill the bacteria already present. It is also essential for food  
15   manufacturers, packers, distributors and retailers to reduce and control the levels of bacteria in food products, especially of the pathogens, in order to prevent illness or death in the ultimate consumer of that food product. Pathogens present a significant risk to human health and can result in food borne illness, and in some circumstances, death. The term "food borne illness" generally refers to illnesses which are caused by  
20   microorganisms consumed by eating any type of food. The Centers for Disease Control (CDC) estimates that food borne illnesses affect a growing number of people every year, with an increasing number of deaths resulting from these illnesses. Food companies are constantly striving to implement antimicrobial techniques that will reduce the risk of harmful bacterial pathogens reaching the consuming public. When people complain of  
25   "food poisoning," they may actually have been exposed to the microorganisms that cause food borne illness. Microbes, bacteria and pathogens are other terms used to describe the microorganisms that cause food borne illness.

          There are, of course, bacteria which are harmless to human health, or are beneficial and are even used in food processing. In addition, other forms of bacteria are  
30   utilized to combat disease. However, bacteria account for more than two-thirds of all outbreaks of food borne illness in the United States. There are literally thousands of

- 2 -

different strains of bacteria that can cause food borne disease; fewer than 50 to 100 are responsible for most of the illnesses.

When encountering the harmful pathogens, food borne illnesses such as infections can result from the ingestion of significant quantities of contaminants that have reproduced in the food itself, or in the small intestines once consumed. This reproduction of the bacteria can, to some extent, be controlled through proper handling of the food during the manufacturing process, as well as proper cooking and food preparation. Thorough cooking can eliminate most of the bacteria present in the food. The problem remains, however, that cooking in food service and at home is very imprecise in terms of accuracy of temperature control and monitoring, and thus food is not always cooked properly. In addition, food is not always stored or handled properly by the food preparer, with such marginal handling, storage and cooking procedures ultimately leaving harmful pathogens in the food product which is to be consumed. Particularly problematic is undercooked meat, more particularly ground beef, which is a common vehicle for infections.

Further problems facing the food industry are the financial and qualitative limitations associated with food products due to food spoilage bacteria present therein which can adversely affect and limit the shelf life of the food products. Over the years, this problem has been addressed by the utilization of various additives which have been incorporated into food products. However, a number of these food additives adversely affect the quality of the food products, as by diminished taste, texture and/or appearance of the treated products.

There is, therefore, a need to provide a method and product which effectively provides an antimicrobial effect on harmful bacterial pathogens found in food products without adversely affecting the quality thereof, to thereby reduce to safe levels the occurrence of harmful bacterial pathogens in such food products. There is, further, a need to provide a method and product which provides an antimicrobial effect on food spoilage bacteria for effectively extending the shelf life of food products without diminishing the quality thereof.

### Summary of the Invention

It is, therefore, an object of the present invention to provide a composition and method for producing an antimicrobial effect in food products in order to control and reduce harmful bacterial pathogens in the food products so that associated health risks are reduced.

It is a further object of the present invention to provide a composition and method for producing an antimicrobial effect in order to control and reduce harmful bacterial pathogens in food products without significantly affecting the flavor, color, texture or odor thereof, so that the quality of the treated food products will not be diminished.

It is another object of the present invention to provide a composition and method for producing an antimicrobial effect by inhibiting the growth of food spoilage bacteria and thereby prolong the shelf life of food products, so that the quality and value of food products can be retained for an extended period of time, and so as to extend the time period during which the color of the food product is maintained, without adversely affecting the quality of the treated food product.

It is an additional object of the present invention to provide an antimicrobial composition and method for producing an antimicrobial effect whereby the composition can be directly applied to food products, so that enhanced antimicrobial activity can be achieved.

It is still a further object of the present invention to provide an antimicrobial composition and method for producing an antimicrobial effect by inhibiting the growth of harmful pathogens and food spoilage bacteria, whereby the composition can be used with raw meat and poultry products.

In order to achieve these and other objects, in accordance with the present invention, an antimicrobial composition and method is provided for reducing the harmful bacterial pathogens present in food products, the method comprising providing a composition having as an active component a mustard seed product or derivative thereof; applying the composition to a food product; blending the composition throughout the food product; allowing the composition to contact the surface area presented by the food product; and creating an antimicrobial effect on the bacteria present in the food product.

- 4 -

There is also provided an antimicrobial composition and method for reducing the harmful bacterial pathogens present in food products, the method comprising providing a composition having as an active component a mustard seed product or derivative thereof, a synthetically produced equivalent thereof, or a combination of a mustard seed product or derivative thereof and a synthetically produced equivalent thereof; applying the composition to a food product; blending the composition throughout the food product; allowing the composition to contact the surface area presented by the food product; and creating an antimicrobial effect on the bacteria present in the food product.

Further, an antimicrobial composition and method is provided for extending the shelf life of food products by reducing and controlling food spoilage bacteria present in food products and thereby maintaining the color and controlling the odor of the food products, which composition includes as an active component a mustard seed product or derivative thereof, a synthetically prepared equivalent thereof, or a combination of a mustard seed product or derivative thereof and a synthetically prepared equivalent thereof; applying the composition to a food product; blending the composition throughout the food product; allowing the composition to contact the surface area presented by the food product; and creating an antimicrobial effect on the bacteria present in the food product.

#### Detailed Description of the Invention

According to the present invention, a composition for producing an antimicrobial effect on food products is provided which comprises an active ingredient, the active ingredient including a mustard seed product or derivative thereof, a synthetically prepared equivalent thereof, or a combination of the two. Due to the antimicrobial tendencies of mustard seed products and derivatives, it has been discovered that its direct addition to raw food products can help to control and reduce the level of bacteria contained within such food product.

The term "mustard seed product" as used herein is defined to include, but is not limited to, a wide variety of mustard seed products and derivatives which are useful in the present invention. Additionally, it is understood that these products can be naturally derived, or can be the synthetic equivalents of such products. These products

- 5 -

include whole mustard seeds, ground mustard seeds, ground mustard bran (the outside coating of the mustard seed), mustard flour (the ground interior of the seed), mustard oil (extracted from the seed), mustard meal (the residual after the mustard oil is extracted from the seed), and isothiocyanates (also extracted from the mustard seed) along with  
5 other enzymes and compounds which are derivatives of mustard seed. As stated above, these products can be the synthetic equivalents of above-listed derivatives, including synthetic or artificial isothiocyanates.

Mustard is a member of the Crucifer family, brassica genus. Some of the more common types of mustard seed include Brassica Nigra (black), Brassica Hirta or  
10 Sinapis Alba (yellow or white), and Brassica Juncea (black, brown or oriental). Of course, this invention is not intended to be limited to the above-listed types of mustard seed. Different varieties of mustard seeds can produce varying amounts of isothiocyanate, thought to be responsible for the antimicrobial effect of the seeds as set forth in more detail below. These variations are dependent upon the different types,  
15 combinations and levels of glucosinolate compounds within the particular variety of the mustard, as well as upon varied growing conditions.

Any other genus associated with the Crucifer family is also useful in accordance with this invention. Some of the plant varieties within this family include turnips, cabbage, brussel sprouts, radishes, rutabaga, broccoli, cauliflower, rape seed,  
20 horse radish, and arabe. It is believed that each of these plants contains a glucosinolate compound and an enzyme capable of activating the glucosinolate.

While the exact antimicrobial process is not completely understood, the following process is suggested. Mustard seeds contain active components, such as an enzyme referred to as myrosinase, along with several compounds referred to as  
25 glucosinolates. The glucosinolates and the enzyme co-exist within the seed without combining, but when allowed to combine in the presence of water they create isothiocyanate, which is believed to be responsible, at least in part, for the antimicrobial activity. One of the antimicrobial isothiocyanates is known as allyl isothiocyanate (AITC), although other compounds within the seed may similarly be active components  
30 which contribute to the antimicrobial effect.

- 6 -

In the preferred embodiment, the composition comprises as an active component the mustard seed extract AITC, discussed above. Preferably, AITC is plated onto a dry carrier and processed into a dry powder. Any conventional carrier can be employed, and can include starch or the like. Specific examples of carriers include maltodextrin and potato starch, although those skilled in the art will recognize that other suitable carriers are equally useful in accordance with this invention. During the plating process, liquid AITC is sprayed onto a dry powder carrier, with the resulting product being a dry powder that has the liquid AITC incorporated therein as an ingredient.

In another embodiment, the composition can include as the active ingredient a starch plated AITC which is encapsulated with fat by conventional means. Specific examples of the fats which can be employed to encapsulate the AITC include animal fats such as beef fat or pork fat, although other suitable fats can be used. In addition, the AITC can also be encapsulated by vegetable oils, or any other appropriate encapsulating products known to those skilled in the art. The AITC is first plated, and is then encapsulated as discussed above to form a resultant dry powder. Such encapsulation delays the release of the AITC, and thereby the antimicrobial effect thereof, until the food product within which it is incorporated is being cooked.

In yet another embodiment of the present invention, the composition comprises encapsulated AITC (or other active component), combined with the non-encapsulated AITC (or other active component), to enhance the antimicrobial action, as the non-encapsulated product controls the bacteria on contact even under refrigeration temperatures, while the encapsulated product provides additional antimicrobial effect as the fat melts and the previously encapsulated AITC is allowed to contact the food product during the cooking process just before the food is consumed. This aids in antimicrobial effectiveness, particularly in situations in which marginal cooking procedures are employed.

The food products which are particularly suited for employing the antimicrobial composition of this invention include a variety of foods and meat proteins, such as raw meats, which can further include beef, pork, poultry, lamb, veal, fish, restructured meats, and processed meats, particularly uncooked processed meats such as fermented sausage. Collectively, these food products are referred to herein simply as



- 7 -

"meat" or "meat products." Such terms are not intended to be limiting, as other food products plagued by bacterial pathogens can be aided by this invention. Particularly suited for the antimicrobial composition and process of the present invention are ground meats, whereby the grinding process further distributes the composition within the meat product to maintain contact with and thereby control surface bacteria which are also incorporated into the meat during the grinding process. In addition, restructured meats are also particularly suited for the antimicrobial composition and process of the present invention, as the composition which has coated the surface of the meat pieces maintains contact with the surface bacteria which ultimately is incorporated into the restructured meat product as the pieces are bound together, shaped and formed.

The composition of the present invention is preferably combined with the food product to deliver a concentration of the active component, for example AITC, ranging from about 50 to 5000 ppm, and more preferably in the range of about 200 to 2500 ppm, and most preferably in the range of about 500 to 2500 ppm. Of course, it is understood that the higher the concentration of active component, the more effective the composition will be, and that the upper boundary of the range is primarily constrained by the need to provide antimicrobial activity without adversely affecting the appearance, taste, color or texture of the meat products. Thus, one skilled in the art will appreciate that if the quality of the meat product is not adversely affected by a particular active component, the effective concentrations can be higher than those set forth above, and such are considered to be within the scope of the present invention.

A method for producing an antimicrobial effect on food products is provided comprising creating a composition including a mustard seed product or derivative thereof, a synthetically produced equivalent thereof, or a combination of a mustard seed product or derivative thereof and a synthetically produced equivalent thereof; applying the composition to a food product; blending the composition throughout the food product to effectively coat the surface area thereof; allowing the composition to contact the surface area presented by the food product and creating an antimicrobial effect on the bacteria present on the food product.

According to the process of the present invention, an antimicrobial composition can be added directly to food products to produce an antimicrobial effect in

- 8 -

the food products, particularly in raw meat products. The composition can be added either as an ingredient in the meat product, or as a product applied to the surface of the meat. In one such method of the present invention, the antimicrobial composition is added to ground meat immediately before, during or immediately subsequent to the grinding process in order to evenly distribute the product throughout the ground meat, killing a large percentage of the bacteria within the meat on contact, including such bacteria as *E. coli*, and inhibiting the growth of the bacteria. In addition, in accordance with another method of the present invention, the composition is added to restructured meat products prior to the shaping and forming of the same. The term "restructured meat" refers to a meat product formed from smaller pieces of typically lower valued meats which are bound together, shaped and reformed to create a more valuable piece of meat. Examples of restructured meat products include chicken fillets and restructured beef tenderloin steaks.

When added to ground or restructured meat, the following process is preferred. Chunks of meat or meat trimmings are provided, and an appropriate amount of the antimicrobial composition, preferably plated AITC, sufficient to achieve the desired concentration of AITC or other active component, is added to the meat, such as by uniformly sprinkling the antimicrobial composition over the meat pieces. The meat pieces with the composition sprinkled thereon are then mixed in a conventional manner for a period of time sufficient to evenly distribute the powder over the surface of the meat pieces. Conventional food mixers are appropriately used for this procedure. Optionally, this may be followed by a holding time for additional antimicrobial activity prior to subsequent processing. This time can range from approximately one to 30 minutes. However, longer periods of time may be appropriate, such as when the food product is stored prior to subsequent processing. The coated meat pieces are then processed by grinding or restructuring by conventional means. Because microorganisms are generally present only on the exposed surfaces of meat pieces, the antimicrobial composition is applied to the meat pieces prior to grinding or restructuring. As the meat pieces are ground or restructured, the composition remains in contact with the surface bacteria that is ground or restructured therein. Thus, in accordance with the present invention, application of the composition prior to grinding or restructuring dramatically increases

the effective concentration of the antimicrobial composition on the potentially contaminated meat surfaces, thereby dramatically increasing the antibacterial activity.

In a second method of the present invention, the composition can be processed into a slurry or other sprayable form for spraying directly on the carcass or large portions of meat, including primal or subprimal sections, such as at the processing plant, or just prior to packaging or freezing. Bacteria are present only on the outer surface of the meat pieces or poultry carcass, and thus such a method of treatment can be effective for producing an antimicrobial effect which will ultimately create a safer end product. Alternatively, still another method of the present invention contemplates processing the composition into a liquefied form as by the addition of a large quantity of water thereto. The composition is then introduced into a vat or other container, and the carcass (or other large portion) of the meat or poultry product is dipped therein prior to packaging, freezing or further processing of such products. Other applications of this invention contemplate use of the composition with fish products, including spraying the composition onto the fish prior to packaging, processing or freezing.

Thus, according to the method and teachings of the present invention, the antimicrobial characteristics of the composition, when combined with meat and poultry products, as well as with other food products, can achieve within these food products a much needed antimicrobial effect, controlling a number of known harmful or food spoilage bacteria or other pathogen strains. In addition to the multiple known strains of *E. coli* which are vulnerable to the antimicrobial effect of this invention, the composition used in accordance with the methods of this invention is also useful for killing and inhibiting the growth of *Streptococcus faecalis*, *Shigella flexneri*, *Hafnia alvei*, *Enterobacter aerogenes*, *Serratia marcescens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Klebsiella pneumoniae*, multiple strains of *Listeria*, and multiple strains of *Salmonella*. Of course, this list is intended to be illustrative, and is not intended to limit the scope of microorganisms against which this invention is useful. Further, it is believed that AITC or other active ingredient in the composition will likewise control multiple types of yeasts, molds, and fungi.

As discussed above, the composition of the present invention is also useful against food spoilage bacteria. This, coupled with the antioxidant effect in mustard seed

- 10 -

products, as well as their derivatives and synthetic equivalents, tends to enhance the shelf life of food products. In addition, this invention is useful in maintaining the color of a food product, such as a meat product, thereby further enhancing the shelf life of the product.

5           According to a further method of the present invention, an initial antimicrobial action occurs when the composition is first mixed with the meat or other food product, and a secondary action occurs during the cooking process as the increased temperature combines with the composition to create additional antimicrobial activity. In the method of this embodiment, the same general process is employed as set forth  
10   above for combining the composition with the meat product. After fine grinding of the meat incorporating the antimicrobial composition, the meat is then cooked in patties or other appropriate form. As the meat cooks, the antimicrobial effect of the composition is enhanced. Particularly useful in accordance with this method is the antimicrobial composition which comprises encapsulated AITC, either alone or in combination with  
15   non-encapsulated AITC. In this manner, the non-encapsulated AITC achieves an initial antimicrobial effect upon direct contact with the meat product, as described above, and upon cooking, the encapsulated AITC achieves an additional antimicrobial action as the fat surrounding the AITC melts, and the AITC incorporated therein is then allowed to directly contact the meat within which it is situated.

20           The following examples are intended to be illustrative of the present invention, but are not intended to be limiting.

#### **Comparative Example 1**

Two compositions were prepared comprising two different types of mustard seed products. The first type of mustard seed product comprised AITC plated  
25   onto starch to form a dry powdered or granular product. This first product was prepared in two concentrations, a starch-maltodextrin plated extract with 7% available AITC, and a starch plated extract with 14% available AITC. The second type of mustard seed product comprised AITC plated onto starch and then encapsulated by fat. This product was prepared as a fat-encapsulated/starch plated extract with 7% available AITC.  
30   Trimblings such as those used to make ground beef were provided. A portion of the beef

- 11 -

was removed for use in evaluating the natural flora present therein, and the remaining beef was inoculated with approximately  $4 \log_{10}$  cfu/g of a five strain mixture of *E. coli* O157:H7 stationary phase culture and allowed to stand for 18 to 24 hours at 2° C. From each of these two portions, a control was prepared which consisted of a segment of the meat treated with potato starch only, without any AITC or other active component incorporated therein. The 7% starch/plated AITC was added to a first part of both the inoculated and the non-inoculated beef trimmings by sprinkling the same as uniformly as possible to achieve a concentration of approximately 500 ppm AITC with respect to the beef as Samples 1a and 1b, and the 14% starch/plated AITC was added to a second part of the beef portions to achieve a concentration of 500 ppm AITC as Samples 2a and 2b. The encapsulated AITC was also sprinkled uniformly on third and fourth parts of both the inoculated and the non-inoculated beef to achieve concentrations of 500 ppm and 2200 ppm AITC with respect to the beef, as Samples 3a, 3b and 4a, 4b, respectively. The meat trimmings with the plated AITC sprinkled thereon were then mixed in a conventional food mixer for a total of two minutes to evenly distribute the powder over the surface of the meat pieces. These coated meat pieces were held for approximately 30 minutes, and were then coarse ground by conventional means. Each of the above samples were packaged and stored for a total of seven days. A first segment of each of the Samples 1a-4a and 1b-4b and of the controls was stored at 4°C, and a second segment was stored at 11°C. Samples 1a-4a and 1b-4b and the controls were evaluated at intervals during storage for total microbial levels of the natural food spoilage flora, and of *E. coli*. After four days, the samples were then fine ground by conventional means. Further readings of the total microbial levels were taken at days 5 and 7. The results of these tests are summarized below in Tables 1-8.

25

Table 1  
Population of *E. coli* O157:H7 ( $\log_{10}$ cfu/g) in  
Inoculated Ground Beef Held at 4°C

30

Day	Control	Sample 1a	Sample 2a	Sample 3a	Sample 4a
0	4.5	4.2	4.2	4.3	3.8
4	4.3	3.9	3.8	4.0	3.6
5	4.1	3.8	3.9	3.8	3.0
7	3.9	3.7	3.6	3.7	3.1

- 12 -

Table 2  
Population of *E. coli* O157:H7 ( $\log_{10}$ cfu/g) in  
Inoculated Ground Beef Held at 11°C

Day	Control	Sample 1a	Sample 2a	Sample 3a	Sample 4a
0	4.5	4.2	4.2	4.3	3.8
4	5.8	3.9	5.3	4.0	3.4
5	5.8	3.8	5.3	3.8	3.3
7	5.8	3.7	5.0	3.7	3.0

Table 3  
Total Aerobic Plate Counts ( $\log_{10}$ cfu/g) in Ground Beef Held at 4°C

Day	Control	Sample 1b	Sample 2b	Sample 3b	Sample 4b
0	2.0	2.6	2.0	3.5	4.2
2	2.6	2.7	2.0	2.9	1.7
4	2.8	2.3	1.9	2.9	1.7
5	3.1	1.2	1.8	2.6	1.5
7	4.6	2.1	2.4	2.5	1.7

Table 4  
Total Aerobic Plate Counts ( $\log_{10}$ cfu/g) in Ground Beef Held at 11°C

Day	Control	Sample 1b	Sample 2b	Sample 3b	Sample 4b
0	2.0	2.6	2.0	3.5	4.2
2	4.8	2.8	3.3	3.3	1.9
4	7.0	4.9	5.2	5.0	2.1
5	8.1	6.2	6.4	5.3	4.2
7	8.2	7.4	6.6	6.4	5.4

Table 5  
Lactic Acid Bacteria Counts ( $\log_{10}$ cfu/g) in Ground Beef Held at 4°C

Day	Control	Sample 1b	Sample 2b	Sample 3b	Sample 4b
0	0.7	1.0	0.7	1.7	2.5
2	1.0	<0.7	0.7	1.3	<0.7
4	3.1	1.8	2.3	2.3	2.5
5	3.0	2.2	1.8	1.9	2.5
7	4.4	0.7	2.2	2.2	1.8

- 13 -

Table 6  
Lactic Acid Bacteria Counts ( $\log_{10}\text{cfu/g}$ ) in Ground Beef Held at 11°C

Day	Control	Sample 1b	Sample 2b	Sample 3b	Sample 4b
0	0.7	1.0	0.7	1.7	2.5
2	4.8	2.3	2.1	2.9	0.7
4	7.0	4.9	5.2	4.9	2.0
5	8.0	6.3	6.3	5.8	3.1
7	7.7	7.0	6.1	6.3	5.9

Table 7  
Psychrotrophic Populations ( $\log_{10}\text{cfu/g}$ ) in Ground Beef Held at 4°C

Day	Control	Sample 1b	Sample 2b	Sample 3b	Sample 4b
0	2.1	3.2	2.5	3.4	4.2
2	2.7	3.0	2.7	3.1	1.4
4	3.0	2.9	2.7	3.7	2.1
5	3.3	2.6	2.1	3.3	1.7
7	4.5	2.8	3.0	4.6	1.5

Table 8  
Psychrotrophic Populations ( $\log_{10}\text{cfu/g}$ ) in Ground Beef Held at 11°C

Day	Control	Sample 1b	Sample 2b	Sample 3b	Sample 4b
0	2.1	3.2	2.5	3.4	4.2
2	6.6	3.1	3.6	3.8	1.2
4	7.3	5.2	6.1	6.4	3.3
5	8.3	7.0	7.2	8.2	5.1
7	8.4	8.3	7.8	8.5	8.1

## 25 Comparative Example 2

The ground beef and antimicrobial composition samples were formulated using plated AITC and encapsulated AITC products, and the general methods as set forth in Example 1. The composition used in this example included a starch plated extract with 14% available AITC, and a fat-encapsulated/plated extract with 7% available AITC.

30 Beef trimmings used to make ground beef were provided. All of the beef was inoculated with a five strain mixture of stationary phase *E. coli* O157:H7 cultures to a level of approximately  $7 \log_{10} \text{cfu/g}$ . This inoculated beef was allowed to stand for 18 to 24 hours at 2°C. In the same manner as above, a control segment was combined with potato starch, and the remaining beef trimmings were combined with the plated AITC by

35 uniform sprinkling to achieve concentrations of approximately 500 ppm and 2200 ppm

- 14 -

AITC with respect to the beef, Samples 5 and 6, respectively. Beef trimmings were also combined by uniform sprinkling with the encapsulated AITC to achieve concentrations of approximately 500 ppm and 2200 ppm AITC with respect to the beef, Samples 7 and 8, respectively. The meat trimmings with the AITC composition sprinkled thereon were then mixed in a conventional food mixer for a total of two minutes to evenly distribute the powder over the surface of the meat pieces. These coated meat pieces were held for approximately 30 minutes, and were then coarse ground by conventional means. Samples 5-8 were then packaged and stored for a total of 4 days at 4°C. Readings were taken to determine microbial levels of the Samples and the control initially, and on day 4 after fine grinding. On day 4, the Samples and controls were fine ground and patties were prepared from each. The patties were cooked on day 5 on an electric skillet to internal endpoint temperatures of 130, 140, 150 and 160°F measured at the geometric center of the patties using a rapid response type K thermocouple probe. Two patties (patty A and patty B) were measured from each sample at each temperature. Prior to cooking and at each cooking temperature, levels of *E. coli* were noted and log reductions in recoverable *E. coli* populations were determined and compared among mustard types and concentrations. The results of these tests are summarized in Table 9 below.

Table 9  
*E. coli* O157:H7 Population ( $\log_{10}$ cfu/g) in Ground Beef  
 Patties Cooked to an Endpoint Temperature

Endpoint Temp (F)	no cook	no cook	130°	130°	140°	140°	150°	150°	160°	160°
	Patty A	Patty B	Patty A	Patty B	Patty A	Patty B	Patty A	Patty B	Patty A	Patty B
Control	6.01	5.88	5.26	5.28	5.44	5.41	3.90	4.53	3.80	3.62
Sample 5	5.37	5.49	4.72	4.77	4.01	2.34	3.73	4.32	NEG.**	4.05
Sample 6	5.31	5.46	3.88	4.03	POS.*	1.40	NEG.**	NEG.**	2.61	NEG.**
Sample 7	5.79	5.76	5.34	5.17	4.69	4.36	2.80	3.11	NEG.**	NEG.**
Sample 8	5.70	5.47	3.56	3.16	1.00	1.54	NEG.**	NEG.**	NEG.**	NEG.**

\* "POS." means *E. coli* O157:H7 was detected by enrichment techniques but below the detection limit of 5 cfu/g ( $0.7\log_{10}$ cfu/g).

\*\* "NEG." means no *E. coli* O157:H7 was detected through direct plate and enrichment techniques.



**Example 3**

Chunks of meat or meat trimmings are provided. An antimicrobial composition is prepared comprising as its active component a combination of AITC extracted from mustard seed and of synthetically prepared AITC, both plated into a dry powder as discussed above. The composition is sprinkled uniformly over the surfaces of the meat to coat the surfaces and achieve a concentration of AITC with respect to the meat of 2500 ppm. The meat pieces with the AITC composition sprinkled thereon are then mixed in a conventional manner using a conventional food mixer for a period of time sufficient to evenly distribute the powder over the surface of the meat pieces. The meat and AITC composition mixture is held for approximately 10 minutes. The coated meat pieces are then ground by conventional means to produce ground meat.

**Example 4**

The meat chunks and antimicrobial composition are combined according to the process of Example 3, but the composition comprises as the active ingredient synthetic AITC. The synthetic AITC can be plated or encapsulated, or a combination of the two. A sufficient amount of the composition is combined with the meat product to achieve a concentration of AITC with respect to the meat of 1500 ppm.

**Example 5**

Chunks of meat or meat trimmings are provided. An antimicrobial composition is prepared comprising as its active ingredient a combination of AITC extracted from mustard seed and plated into a dry powder as discussed above, and synthetically prepared AITC. The composition is uniformly sprinkled over the surfaces of the meat to coat the surfaces and achieve a concentration with respect to the meat of 800 ppm AITC. The meat pieces with the AITC composition sprinkled thereon are then mixed in a conventional manner using a conventional food mixture for a period of time sufficient to evenly distribute the powder over the surface of the meat pieces. The coated meat pieces are then processed by binding, shaping and forming to make a restructured meat product.

In alternative embodiments of this invention, spices other than mustard seed product are useful. In order for these spices to be of particular usefulness in this invention, they should have essential oils which have antimicrobial tendencies. Some of

- 16 -

the more effective of these spices include garlic, onion, and clove products. Other spices exhibiting a lesser degree of antimicrobial effect include ginger, basil, cumin, and coriander. It has been found, and is within the scope of this invention, that an enhanced antimicrobial effect may exist in the combination of mustard or its derivative, or the  
5 synthetic equivalent thereof, with one or more of these or other spices, or with other flavoring ingredients (such as liquid smoke) to cause the desired antimicrobial effect.

From the foregoing, it will be seen that this invention is one well adapted to attain all the ends and objects hereinabove set forth together with other advantages which are obvious and which are inherent to the method and composition.

10 It will be understood that certain features and subcombinations are of utility and may be employed without reference to other features and subcombinations. This is contemplated by and is within the scope of the claims.

Since many possible embodiments may be made of the invention without departing from the scope thereof, it is to be understood that all matter herein set forth is  
15 to be interpreted as illustrative and not in a limiting sense.

- 17 -

Claims

Having thus described the invention, what is claimed is:

1. A method for producing an antimicrobial effect on food products, said method comprising: providing an antimicrobial composition having an active ingredient  
5 comprising a mustard seed product or derivative thereof, a synthetically prepared equivalent thereof, or a combination of the same; applying the composition to a food product; blending the composition throughout the food product; allowing the composition to contact the surface area presented by the food; and creating an antimicrobial effect on bacteria present in the food product.
- 10 2. The method as set forth in claim 1, wherein the active ingredient includes an isothiocyanate compound.
3. The method as set forth in claim 2, wherein the isothiocyanate compound includes allyl isothiocyanate.
- 15 4. The method as set forth in claim 3, wherein the allyl isothiocyanate is extracted from mustard seed.
5. The method as set forth in claim 3, wherein the allyl isothiocyanate is synthetically prepared.
6. The method as set forth in claim 1, wherein the applying step comprises sprinkling the composition onto the food product.
- 20 7. The method as set forth in claim 1, wherein the applying step comprises spraying the composition onto the food product.
8. The method as set forth in claim 1, wherein the applying step comprises dipping the food product into a container containing the composition.
9. The method as set forth in claim 1, wherein the bacteria includes  
25 food borne spoilage and pathogenic microorganisms.
10. The method as set forth in claim 1, wherein the food product is raw meat.
11. The method as set forth in claim 10, further comprising the step of grinding the raw meat and antimicrobial composition mixture to form ground meat.

12. The method as set forth in claim 10, further comprising the step of preparing a restructured meat product from the raw meat and antimicrobial composition mixture.

13. The method as set forth in claim 1, wherein the active component  
5 is plated onto a carrier.

14. The method as set forth in claim 1, wherein the active component is plated onto a carrier and encapsulated.

15. An antimicrobial composition for adding to food products to produce an antimicrobial effect on the food products, the composition comprising an  
10 active ingredient comprising a mustard seed product or derivative thereof, a synthetic equivalent of the mustard seed product or its derivative, or a combination of the two.

16. The composition as set forth in claim 15, wherein the active ingredient is plated onto a carrier.

17. The composition as set forth in claim 16, wherein the plated active  
15 ingredient is encapsulated.

18. The composition as set forth in claim 15, wherein the active ingredient is an isothiocyanate compound.

19. The composition as set forth in claim 18, wherein the isothiocyanate compound is allyl isothiocyanate.

20. The composition as set forth in claim 15, wherein the food product  
20 is raw meat.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01784

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A23B4/20 A23L3/3472 A23L3/3535

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A23B A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 557 946 A (GREEN CROSS CORP) 1 September 1993	1-7, 9, 15, 18, 19
Y	see abstract  see page 2, line 1 - line 10 see page 3, line 20 - page 4, line 15 see claims	8, 10-14, 16, 17, 20
Y	EP 0 312 519 A (MONSANTO CO) 19 April 1989 see page 3, line 1 - line 9	8

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 May 1998

Date of mailing of the international search report

26/05/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Boddaert, P

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01784

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 017, no. 221 (C-1054), 7 May 1993 -& JP 04 357953 A (NOBORU YAMADA; OTHERS: 01), 10 December 1992,	15, 16, 18, 19
A	see abstract  -& DATABASE WPI Derwent Publications Ltd., London, GB; AN 93-031289 XP002064139 see abstract	1-4, 13, 14, 17
X	--- PATENT ABSTRACTS OF JAPAN vol. 096, no. 005, 31 May 1996 -& JP 08 012511 A (NAKANO VINEGAR CO LTD), 16 January 1996,	1-4, 15, 18, 19
Y		13, 14, 16, 17
A	see abstract -& DATABASE WPI Derwent Publications Ltd., London, GB; AN 96-112581 XP002064140 see abstract	5-10, 20
X	--- PATENT ABSTRACTS OF JAPAN vol. 006, no. 186 (C-126), 22 September 1982 -& JP 57 099182 A (UEDA SEIYU KK), 19 June 1982,	1-4, 15, 18, 19
A	see abstract  -& DATABASE WPI Derwent Publications Ltd., London, GB; AN 82-62576E XP002064141 see abstract	5-12, 16, 17, 20
X	--- DATABASE FSTA INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANKFURT/MAIN, DE Federation of European Chemical Societies, 1993 DRDAK M ; RAJNIAKOVA A BUCHTOVA V: "Mustard phytoncides utilization in the process of food preservation" XP002064138	1-4, 9, 15, 18, 19
Y	FSTA - AN - 93-1-12-s0053 Nutrient Bioavailability Symposium	10-12, 20
A	see abstract	5-8, 16, 17
	--- -/--	

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01784

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HERMEY B ; LUDI R: "Characteristics of various types of mustard seed and their use in food manufacture" FLEISCHEREI, vol. 45, no. 11, 1994, pages 46-50, XP002064137	1-4, 10-12, 15, 18-20
A	see the whole document	5-9, 13, 14, 16, 17

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/01784

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0557946 A	01-09-1993	AT 152878 T	15-05-1997
		AU 665229 B	21-12-1995
		AU 3373593 A	02-09-1993
		CA 2090172 A	27-08-1993
		CN 1076332 A	22-09-1993
		DE 69310571 D	19-06-1997
		DE 69310571 T	13-11-1997
		JP 6192018 A	12-07-1994
		US 5703124 A	30-12-1997
EP 0312519 A	19-04-1989	US 4770884 A	13-09-1988
		CN 1031931 A	29-03-1989
		JP 1039966 A	10-02-1989
		PT 87891 A	01-08-1988